Identification of genomic regions harboring diversity between Holstein and two local endangered breeds, Modenese and Maremmana

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A B S T R A C T

The preservation of genetic diversity in farm animals is important to cope with future challenges like climate changes and different production environments. The knowledge of the genes making the endangered breeds different from the more productive breeds, responsible of their decline, might contribute to support the "in situ" on-farm conservation, which is considered the most favorable means to preserve genetic material at risk.

In the present study, three genome-wide breed-to-breed comparisons, using about 30,000 Single Nucleotide Polymorphisms, allowed the identification of markers with the highest difference in allele frequency between Holstein and two Italian endangered cattle breeds of Podolian origin: the dairy Modenese and the suckler, rustic Maremmana. For most of the genes either encompassing or in proximity of these markers the influence on some important economic traits in cattle had been already described in literature. The majority of differentiation signals between each of the two endangered and the Holstein are related to genes influencing lactation traits, carcass characteristics, resilience and disease resistance.

1. Introduction

The preservation of diversity in farm animals is important for the future of agriculture and food industry; "in situ" on-farm conservation is considered the most favorable means to preserve genetic material (Oldenbroek, 2007). Conservation actions are generally based on the effective population size (FAO, 2000) by classifying the breeds as critical, endangered and at risk. However, decision-making for breed conservation has not considered, to date, the potential candidate genes in which the endangered livestock might have preserved useful alleles. In the present study, by exploiting DNA marker panels, a genome-wide analysis of two Italian endangered, low productive breeds was performed with the aim to identify selection sweeps in their genome influencing economic traits which might justify their conservation "in situ".

The two endangered breeds, Modenese and Maremmana, have been almost fully substituted by more productive breeds, mainly the Holstein. They have Podolian ancestors in common: Modenese originated from the reddish-coat cow population farmed in the area of Carpi (Modena, Northern Italy) in middle IX century, with influences from Podolian cattle (grey coat), through some selection for milk, meat and for a white coat (Hiemstra et al., 2010); the origin of Maremmana can be traced back to the Etruscan era when local cows were crossed with Podolian cattle (Maretto et al., 2012). The Modenese is a dual-purpose breed producing only 5265 ± 1148 kg milk in 309 days (Petrera et al., 2016); although this milk is more suitable for the production of Parmesan cheese, due to a fat/protein ratio close to 1 (AIA, 1981–2002) and to higher values of casein and titrable acidity (Petrera et al., 2016), less than 1000 cows were left in 1998 (FAO, 2000) while around 1930 population size was 230,000 animals (CNR, 1983).

The Maremmana owes the name to the Maremma, a coastal region of Italy between southern Tuscany and northern Latium, which was marshy land one century ago; in 1944 this area hosted 288,000 animals of this breed (CNR, 1983), followed by a decrease to 44,000 in 1983. After mechanization in agriculture and drainage of the marshy area, the Maremmana began to face a crisis, getting close to extinction at the end of the 1980's (FAO, 2002). Following the implementation of agri-environmental measures (EU Regulation 2078/92), incentives were introduced to protect animals threatened of extinction, and the number has since increased to over 10,000 registered animals in the Herdbook in 2017 (www.anabic.it/index1.htm). Maremmana is a suckler cow, with excellent mothering abilities and sufficient milk for weaning two calves on range conditions (Moioli et al., 2017).

Following the proposals of Bonin et al. (2007) who emphasized the relevant importance, for conservation, of loci under selection, because they reveal functional adaptation, aim of this study was to detect loci differentiating Modenese and Maremmana from the Holstein breed, having the latter played an important role in their decline, and to identify potential candidate genes determining their peculiarities. Whether genetic differentiation has been the consequence of selection or of genetic drift will not modify the implications of the present study.

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2. Materials and methods

DNA of 47 Modenese, 138 Holstein and 124 Maremmana cows was genotyped using the BovineSNP54K BeadChip (Illumina, San Diego, CA, USA) which contains 54,524 single nucleotide polymorphisms (SNPs). All cows were registered in the Herdbooks of the corresponding breeds. The majority of Holstein cows belonged to various herds of six districts in the most specialized dairy farming area of Northern Italy; in one these farms Modenese cows were also raised. Twenty-two Holstein cows belonged to the experimental farm of the Animal Production Research Centre in Monterotondo (CREA), near Rome in Italy, where also the Maremmana cows were maintained.

Genotypic data obtained for the three breeds were used in a genome-wide analysis in pairwise breed-to-breed comparisons. Markers for missing data ($\geq 5\%$) and minor allele frequency (MAF) ≤ 0.01 were excluded. Call rate for samples was also computed and cows with more than 10% missing data were removed.

To detect genomic regions that may have been under positive selection, the hypothesis that SNP effect were $\neq 0$, chi^2 values for each marker were calculated, in pairwise breed comparisons, with the GenABEL package in R (Aulchenko et al., 2007). The "qtscore" function was used by adopting the "binomial" option, i.e. indicating the two breeds by 0/1.

To correct for multiple testing, the Bonferroni correction was applied to the P-values of each SNP, using the Proc Multtest of SAS software (SAS, 2007). Manhattan plots for each breed pair were built up indicating, on the Y axis, -log10 of the Bonferroni corrected P-value of each SNP with respect to the genomic position.

To identify the putative candidate genes explaining the variability between the breeds, only the SNPs displaying the highest difference in allele frequency between each breed pair (>0.75) were used to retrieve the annotated genes from the NCBI database. The genomic region around these SNPs (± 100 kb up- or downstream) was examined on the UMD 3.1.1 genome assembly (https://www.ncbi.nlm.nih.gov/; annotation release 104).

To investigate whether the different allele frequency of the significant SNPs between breed pairs were either the evidence of positive selection signatures for the improvement of an economic trait, or DNA signals preserved from the indicine origin, the genes in proximity of the SNPs were searched in the relevant literature, and only if an author had reported the influence of one of these genes on a trait in cattle, the genes were classified in genes affecting milk traits, carcass traits, functional traits (fertility, SCC) or adaptation, the latter including resistance to diseases and heat stress as well as genomic divergence of zebu and taurine cattle.

Functional annotation tool of PANTHER classification System (Version10.0, Mi et al., 2013) was used to inspect Gene Ontology (GO) terms mapping to protein-coding genes and assign the genes to the three main categories, Biological Process (BP), which includes broad biological goals; Molecular Function (MF), which describes the tasks performed by individual gene products and Cellular Component (CC), which involves subcellular structures, locations, and macromolecular complexes.

3. Results

After filtering, the number of markers used in the search for putative candidate genes were 38,899 (Holstein-Maremmana), 35,113 (Holstein-Modenese) and 38,298 (Modenese-Maremmana). Seventeen samples were removed because of low genotyping rate: one Modenese, 4 Holstein and 12 Maremmana.

Genome-wide Manhattan plots displaying the Bonferroni corrected chi^2 P-values for each SNP with respect to their genomic position were shown in Fig. 1 for the three pairwise comparisons; a: Holstein-Maremmana, b: Holstein-Modenese, c: Modenese-Maremmana. The significant SNPs after the Bonferroni correction (P-value < 0.0000001)
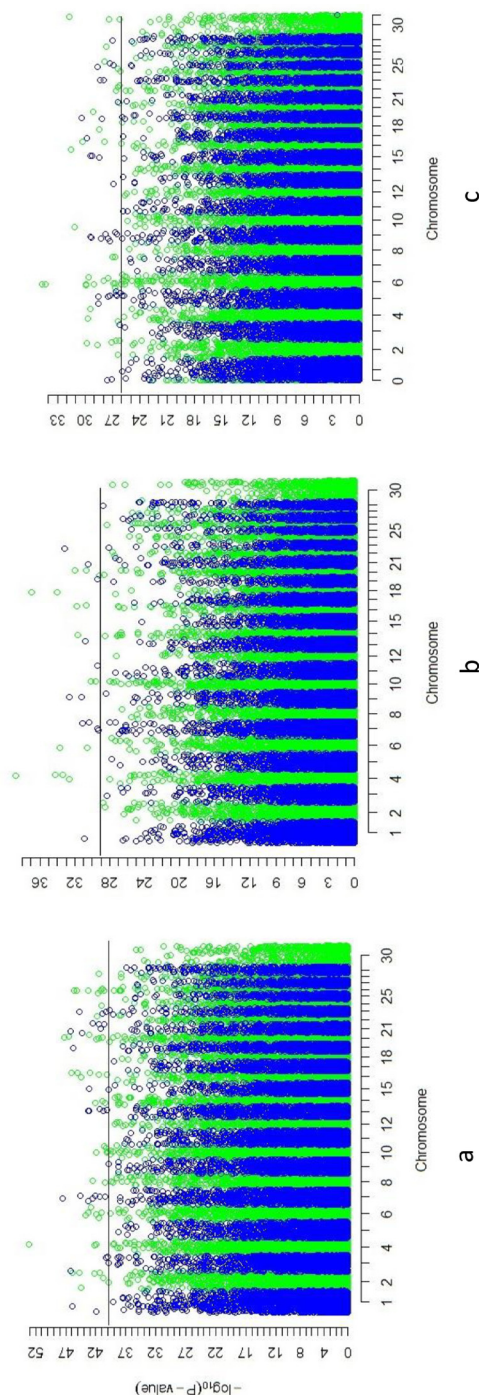

Fig. 1. Genome-wide Manhattan plots: (a) Holstein-Maremmana, (b) Holstein-Modenese, (c) Modenese–Maremmana.

are above the black line.

The SNPs differing in allele frequency > 0.75 between each pair of breeds are reported in the supplementary Tables S1, S2 and S3. The majority of them (80) were detected in the Holstein-Maremmana comparison (Table S1) while less signals (33) differentiated Holstein from Modenese (Table S2). The signals differentiating Modenese from Maremmana were 76 (Table S3). The Tables report, for each significant SNP, chi^2 values, raw and Bonferroni-adjusted chi^2 P-values, reference allele, frequency of this allele in each breed and the list of annotated genes located in the genomic region around these SNPs (± 100 kb up- or downstream).

In the comparison Holstein-Maremmana, monomorphic SNPs were 1 for the first and 5 for the second breed. In the comparison Holstein-Modenese, monomorphic SNPs were 1 for the first and 2 for the second breed. In the comparison Modenese-Maremmana, monomorphic SNPs were 8 for the first and 4 for the second breed.

Two significant SNPs - ARS-BFGL-NGS-116590 (chromosome 4, position 77635835) and BTA-79025-no-rs (chromosome 7, position 47892300) were detected in the comparison of Holstein with both the Maremmana and the Modenese. Two SNPs on chromosome 6 - Hapmap27072-BTC-033816 and Hapmap27503-BTC-033786 were detected in the comparison of Modenese with both the Maremmana and the Holstein. One SNP - Hapmap49746-BTA-76106, on chromosome 6, position 46140090 – was detected in the comparison of Maremmana with both the Holstein and the Modenese.

In Tables 1–3, all genes of Tables S1–S3 were listed, for each breed pair, with indication of the chromosome position and the putative gene function when described, in cattle, by the literature.

Under the hypothesis that the differences between the examined breeds relied either upon alleles inherited by the Podolian ancestors, and therefore involved in adaptation and functional traits, or upon alleles modified by positive selection for improving milk and beef traits, the genes functions were classified in four groups: 1. adaptation, including the genes differentiating indicine from taurine breeds as well as the genes involved in heat and parasite tolerance and in disease resistance; 2. functional traits, i.e. fertility, longevity Somatic Cell Count (SCC); 3. selection for milk; 4. selection for beef traits. The four groups were arbitrarily inferred to facilitate the discussion, and the frequency of the genes in the four groups, for each breed pair, was reported in Fig. 2.

Table 4 was obtained using PANTHER's functional annotation tool to inspect GO terms mapping to protein-coding genes. Genes were classified in the GO categories BP, MF and CC. The majority of the genes differentiating each of the three breed pairs fell in the same categories: under the BP classification in "cellular process (GO:0009987)", and "metabolic process (GO:0008152)"; under the MF classification in the categories "binding (GO:0005488)" and "catalytic activity (GO:0003824)"; under the CC classification in the categories "cell part (GO:0044464)" and "organelle (GO:0043226)".

Table 1

Annotated genes on the Bos Taurus UMD 3.1.1 (annotation relase 104) in proximity of the SNPs differentiating Holstein from Maremmana, with indication of the closest SNP, the trait influenced by the gene and literature reference.

SNP	CHR	Position	Gene symbol	Economic trait	Citation
ARS-BFGL-NGS-103465	2	61822538	UBXN4	beef	Karisa et al. (2013)
			R3HDM1	beef	Zhao et al. (2015)
ARS-BFGL-NGS-66184	2	122997112	SERINC2	adaptation	Newman et al. (2015)
			NKAIN1	milk	Pareek et al. (2017)
BTA-31250-no-rs	2	128437731	SYF2	milk	Do et al. (2017)
Hapmap51459-BTA-49829	2	132170155	EIF4G3	functional traits	Hoglund et al. (2016)
ARS-BFGL-NGS-3713	3	101785952	TCTEX1D	beef	Lemos et al. (2016)
			PLK3	beef	Zarek et al. (2017)
ARS-BFGL-NGS-23119	3	106412527	SMAP2	milk	Suchocki et al. (2016)
			COL9A2	beef	Taye et al. (2017b)
			ZMPSTE24	milk	Yodklaev et al. (2017)
BTA-88376-no-rs	4	71356324	OSBPL3	beef	De Almeida-Santana et al. (2015)
ARS-BFGL-NGS-116590	4	77635835	NUDCD3	milk	Bertolini et al. (2015)
			NPC1L1	milk	Bertolini et al. (2015)
			TMED4	adaptation	Huang et al. (2012)
Hapmap49746-BTA-76106	6	46140090	LGI2	functional traits	Zhang et al. (2016a)
BTA-79025-no-rs	7	47892300	CAMLG	beef	Randawa et al. (2015)
			DDX46	functional traits	Moore et al. (2015)
			SAR1B	milk	Xu et al. (2014)
			TXNDC15	beef	Randawa et al. (2015)
ARS-BFGL-NGS-30237	8	54105059	GNAQ	beef	Wu et al. (2014)
ARS-BFGL-BAC-11657	10	48926752	RORA	beef	Cesar et al. (2014)
ARS-BFGL-NGS-108215	10	85085819	RBM25	functional traits	Moore (2015)
BTB-01997512	13	47415727	ZMYND11	milk	Zhao et al. (2015)
Hapmap54398-rs29020900	14	21870878	SNTG1	functional traits	Meszaros et al. (2014)
Hapmap31956-BTC-054628	14	26035082	FAM110B	beef	Lee et al. (2013)
ARS-BFGL-NGS-116109	15	34763290	GRAMD1B	beef	Serao et al. (2013)
ARS-BFGL-NGS-101656	16	41992472	DHRS3	beef	Chen et al. (2011)
BTB-00647886	16	59638184	ASTN1	milk	Taye et al. (2017a)
ARS-BFGL-NGS-17554	19	27099600	SPAG7	beef	Cesar et al. (2016)
			MINK1	adaptation	Gautier et al. (2009)
ARS-BFGL-NGS-74334	20	31752546	SEPP1	adaptation	O'Gorman et al. (2009)
			CCDC152	milk	Do et al. (2017)
BTA-113191-no-rs	20	33256096	PLCXD3	milk	Jiang et al. (2014)
ARS-BFGL-NGS-116981	20	39100174	AGXT2	adaptation	Mariasegaram et al. (2007)
ARS-BFGL-NGS-111442	22	52783353	SCAP	milk	Rincon et al. (2012)
ARS-BFGL-NGS-101374	26	21832456	LBX1	milk	Van der Berg et al. (2014)
BTB-00932019	26	21870752	BTRC	milk	Raven et al. (2016)
ARS-BFGL-NGS-5577	26	22215515	MGEA5	milk	Do et al. (2017)
			FGF8	functional traits	Santos-Biase et al. (2012)
ARS-BFGL-NGS-110383	26	22648404	LBD1	adaptation	Kim and Rotschild (2014)

Table 2
Annotated genes on the Bos Taurus UMD 3.1.1 (annotation relase 104) in proximity of the SNPs differentiating Holstein from Modenese, with indication of the closest SNP, the trait influenced by the gene and literature reference.

SNP	CHR	Position	Gene symbol	Economic trait	Citation
ARS-BFGL-NGS-116590	4	77635835	TMED4	adaptation	Huang et al. (2012)
			NPC1L1	mil.	Bertolini et al. (2015)
			NUDCD3	milk	Bertolini et al. (2015)
			CAMK2B	beef	Zarek et al. (2017)
Hapmap27072-BTC-033816	6	38133743	PKD2	beef	Abo-Ismail et al. (2014)
Hapmap27503-BTC-033786	6	38173166	SPP1	milk	Bissonnette (2018)
ARS-BFGL-NGS-54753	6	88210451	SLC4A4	adaptation	Sahana et al. (2014)
ARS-BFGL-NGS-100845	7	42460306	OR6F1	beef	de Almeida Santana et al. (2016)
BTA-79025-no-rs	7	47892300	CAMLG	beef	Randawa et al. (2015)
			DDX46	functional traits	Moore et al. (2015)
			SAR1B	milk	Xu et al. (2014)
			TXNDC15	Beef	Randawa et al. (2015)
ARS-BFGL-NGS-35611	7	98799246	ERAP1	adaptation	Blanco et al. (2017)
			ERAP2	adaptation	Porto-Neto et al. (2013)
			LNPEP	adaptation	Porto-Neto et al. (2013)
ARS-BFGL-NGS-43711	9	87117785	UST	functional traits	Purfields et al. (2015)
BTB-01397485	11	33236456	NRXN1	functional traits	Raven et al. (2016)
ARS-BFGL-NGS-43715	11	86888060	PDIA6	milk	Wu et al. (2013)
			ATP6V1C2	beef	Cohen-Zinder et al. (2016)
ARS-BFGL-NGS-607	14	18460103	ZHX2	beef	Serao et al. (2013)
BTB-00557532	14	24643266	TMEM68	beef	Magalhaes et al. (2016)
ARS-BFGL-NGS-86599	18	14359452	ACSF3	beef	Islam (2009)
Hapmap44238-BTA-42338	18	14401871	ANKRD11	functional traits	Moore et al. (2016)
ARS-BFGL-NGS-78203	19	48725899	CCDC47	beef	Silva-Vignato et al. (2017)
			DDX42	functional traits	Hatzirodos et al. (2014)
			SMARCD2	functional traits	Killeen et al. (2014)
			GH1	beef	Curil et al. (2006)
ARS-BFGL-NGS-118446	23	4083573	COL21A1	milk	Cole et al. (2011)
Hapmap54795-rs29014478	23	10451041	STK38	adaptation	Brand et al. (2011)

4. Discussion

The number of sampled animals is one of the major concerns in studies on breed diversity. Although the sampling of thirty animals per breed is considered sufficient to estimate genetic diversity (Kijas et al., 2012) the relatively small number of Modenese cows available for this study could of course influence the results. Moreover, the inbreeding that might exist in populations of small size is a concern which might affect not only to the results of diversity studies but also conservation actions. Animals of the two endangered breeds here analyzed, Maremmana and Modenese, are registered in the Herdbook which provides directives on the sires to use to maintain an acceptable inbreeding level. These breeds were selected under the hypothesis that they have maintained a potential genetic adaptation because they have not been submitted to intensive selection programs. However, Boettcher et al. (2015) observed that the value of the data of genetic characterization of many breeds of livestock is still questionable for study of adaptation. These authors emphasize the current shortage of specific knowledge about the adaptation of a population to a given environment and whether it could survive elsewhere. In the present study, the discussion of the genes in proximity of the significant SNPs will be focused, on one side, on the potential influence on functional traits and disease resistance, on the other side, on the positive selection to improve milk and beef traits.

In this study, chi^2 test was preferred to the more widely used F_{st} test to detect signals of differentiation between two endangered breeds and the Holstein. In fact, Moioli et al. (2015) showed that chi^2 and F_{st} were highly correlated, but chi^2 has the advantage to produce probability values which are mandatory when comparing population pairs.

The majority of significant SNPs were found in the comparisons of Maremmana with both Holstein (80 SNPs) and Modenese (76 SNPs) indicating that this breed has been scarcely touched by human selection for productive traits (Supplementary Tables S1–S3). The review of the literature citing the genes in proximity of these SNPs will contribute to the understanding of the role they have played in influencing economic traits important for cattle.

4.1. Holstein–Maremmana

For 41 of the 131 genes (Supplementary Table S1) detected in proximity the SNPs differentiating Holstein from Maremmana the literature had described a role in cattle (Table 1, Fig. 2). The majority of the genes were reported among the genes affected by selection to improve milk yield, in evident agreement with the opposite breeding directions of the two breeds. ASTN1, BTRC, NKAIN1, NPC1L1, NUDCD3, SAR1B and ZMYND1 were identified in genomic studies aiming to detect the genes under positive selection for milk yield, and appeared among those which best differentiated the Holstein from other breeds (Taye et al., 2017a; Raven et al., 2016; Pareek et al., 2017; Bertolini et al., 2015; Xu et al., 2014; Zhao et al., 2015). SNPs detected within the SMAP2 and the PLCXD3 genes were reported to affect milk proteins respectively by Suchocki et al. (2016) and Jiang et al. (2014); an association of SCAP with milk fatty acids was reported by Rincon et al. (2012); CCDC152, MGEA5 and SYF2 were associated to lactation persistence by Do et al. (2017); LBX1 was associated to dairy type (van den Berg et al., 2014) and ZMPSTE24 to the age at first calving (Yodklaew et al., 2017).

Differences in meat quality – fatty acid content of muscle and marbling - between Holstein and Maremmana might be described by COL9A2, OSBPL3, R3HDM1, RORA, SPAG7 and TCTEX1D (Taye et al., 2017b; de Almeida Santana et al., 2015; Zhao et al., 2015; Cesar et al., 2014; Lemos et al., 2016); differences in feed efficiency, slaughter weight and carcass yield by CAMLG, TXNDC15, DHRS3, FAM110B, GNAQ, GRAMD1B, PLK3 and UBXN4 (Randawa et al., 2015; Chen et al., 2011; Lee et al., 2013; Wu et al., 2014; Serao et al., 2013; Zarek et al., 2017; Karisa et al., 2013).

LBD1, MINK1, and TMED4 were detected in genomic studies searching for genes differentiating zebu and taurine cattle (Kim and Rothschild, 2014; Gautier et al., 2009; Huang et al., 2012), AGXT2 was associated to heat tolerance (Mariasegaram et al., 2007), SEPP1 to

Table 3

Annotated genes on the Bos Taurus UMD 3.1.1 (annotation relase 104) in proximity of the SNPs differentiating Modenese from Maremmana, with indication of the closest SNP, the trait influenced by the gene and literature reference.

SNP	CHR	Position	Gene symbol	Economic trait	Citation
ARS-BFGL-NGS-115971	1	1385605	*GART*	functional traits	Fritz et al. (2013)
			IFNGR2	adaptation	Killick et al. (2011)
			IFNAR1	adaptation	Killick et al. (2011)
Hapmap47394-BTA-74024	5	75660485	*NCF4*	milk	Raven et al. (2016)
			CSF2RB	milk	Raven et al. (2016)
Hapmap54968-rs29014852	5	81554485	*CCDC91*	adaptation	Kupper et al. (2014)
ARS-BFGL-NGS-5761	5	97490658	*CDKN1B*	functional traits	Abdel-Shafy et al. (2014)
ARS-BFGL-NGS-54496	5	106178425	*FGF6*	milk	Do et al. (2017)
			FGF23	milk	Do et al. (2017)
			TIGAR	adaptation	Kim et al. (2017)
			CCND2	milk	Do et al. (2017)
ARS-BFGL-NGS-116906	5	106538208	*PARP11*	functional traits	Han and Penagaricano (2016)
			CRACR2A	milk	Do et al. (2017)
Hapmap27072-BTC-033816	6	38133743	*PKD2*	beef	Abo-Ismail et al. (2014)
Hapmap27503-BTC-033786	6	38173166	*SPP1*	milk	Bissonnette (2018)
Hapmap27083-BTC-041166	6	38825835	*FAM184B*	beef	Zhang et al. (2016b)
Hapmap23507-BTC-041133	6	38845992	*DCAF16*	beef	Zhang et al. (2016b)
Hapmap31285-BTC-041097	6	38869785	*LCORL*	beef	Zhang et al. (2016b)
Hapmap49746-BTA-76106	6	46140090	*LGI2*	functional traits	Zhang et al. (2016b)
ARS-BFGL-NGS-69759	6	63165597	*ATP8A1*	beef	Taye et al. (2017b)
Hapmap60224-rs29001782	6	85178107	*UBA6*	functional traits	Abdel-Shafy et al. (2014)
BTB-01654826	6	88891318	*NPFFR2*	functional traits	Sahana et al. (2014)
Hapmap41153-BTA-110528	9	10134275	*SMAP1*	milk	Suchocki et al. (2016)
ARS-BFGL-NGS-112742	9	39187022	*FYN*	functional traits	Pinto de Melo et al. (2017)
ARS-BFGL-NGS-11311	15	40612416	*TEAD1*	adaptation	Kim et al. (2017)
Hapmap39381-BTA-118849	16	66543047	*RGL1*	milk	Yodklaew et al. (2017)
BTA-39826-no-rs	16	68811897	*HMCN1*	beef	Abo-Ismail et al. (2014)
BTA-03959-no-rs	18	62221442	*VSTM1*	milk	Bentley et al. (2015)
			NLRP9	functional traits	Cochran et al. (2013)
			EPN1	milk	Suchocki et al. (2016)
ARS-BFGL-NGS-2725	23	24904300	*GSTA2*	functional traits	Pinto de Melo et al. (2015)
Hapmap53349-ss46526376	23	27305227	*EHMT2*	adaptation	Fisher et al. (2007)
			LSM2	milk	Suchocki et al. (2016)
ARS-BFGL-NGS-72290	24	57257505	*NARS*	milk	Li et al. (2016)
ARS-BFGL-NGS-117215	25	22890173	*SLC5A11*	functional traits	Hayashi et al. (2017)
ARS-BFGL-BAC-43226	25	34663848	*MDH2*	beef	Shin and Chung (2016)
Hapmap56389-rs29012404	30	141044156	*TMSB4X*	beef	Chen et al. (2011)
			TLR7	adaptation	Rensetti et al. (2016)
			TLR8	adaptation	Rensetti et al. (2016)

trypanotolerance (O'Gorman et al., 2009) and *SERINC2* to disease resistance (Newman et al., 2015), therefore the last five genes were classified as adaptation genes, likely derived from the Podolian origin of the Maremmana.

The genes influencing functional traits are six. Four of them were reported to influence fertility: in detail, SNPs associated with number of inseminations per conception, interval from calving to first insemination, and 56-day non-return rate were identified within the *EFGF8* gene (Hoglund et al., 2016); a SNP associated with number of oocytes collected from ovum pickup was detected in *FGF8* (Santos-Biase et al., 2012); *DDX46* and *RBM25* were found differentially expressed in Corpus luteum of high vs. low fertile cows (Moore et al., 2015). Finally, *LGI2* and *SNTG1* were associated to longevity (Zhang et al., 2016a; Mészáros et al., 2014). Holstein is known to have been affected by declining fertility and reduced longevity in the past forty years; on the other hand, Maremmana cows are appreciated for the high longevity and fertility. The high longevity of the Maremmana is further emphasized by the fact that *LGI2* gene is in proximity of a SNP (Hapmap49746-BTA-76106) detected also in the comparison Modenese–Maremmana.

Two significant SNPs differentiating Holstein from Maremmana (ARS-BFGL-NGS-116590 and BTA-79025-no-rs) were also significant in the comparison Holstein-Modenese. These SNPs are in proximity of the above discussed genes, influencing either milk traits (*NPC1L1, NUDCD3, SAR1B*), carcass traits *(CAMLG* and *TXNDC15)* or functional traits (*TMED4, DDX46* and *SAR1B*) so confirming Holstein superiority as dairy cow, as well as the better beef attitude and functionality of the other two breeds.

4.2. Holstein–Modenese

The 33 significant SNPs differentiating Holstein from Modenese encompass 61 potential candidate genes (Supplementary Table S2). The literature described the role played in cattle by 29 of them (Table 2, Fig. 2). Almost 40% of them influence beef characteristics, giving evidence of the selection towards a dual-pupose breed of the Modenese. Some of them influence feed efficiency: *CAMK2B, ATP6V1C2* and *ZHX2* were reported to be associated to feed intake (Zarek et al., 2017; Cohen-Zinder et al., 2016; Serao et al., 2013), while *OR6F1* with feed conversion ratio (de Almeida Santana et al., 2016). *PKD2, CAMLG, TXNDC15, CCDC47* and *GH1* influence body size and carcass yield (Abo-Ismail et al., 2014; Randawa et al., 2015; Curi et al., 2006; Silva-Vignato et al., 2017). *TMEM68* and *KAZN* influence meat quality: tenderness and marbling (Magalhaes et al., 2016; Islam, 2009).

Only 20% of the genes influence milk traits, confirming that the principal selection objective is for dairy traits also for the Modenese. *SAR1B* was reported among the genes under positive selection for milk yield (Xu et al., 2014); *NPC1L1* and *NUDCD3* among the genes which best differentiated the Holstein from other breeds (Bertolini et al., 2015). Two more genes (*PDIA6* and *COL21A1*) influence udder conformation and dairy morphology, while *SPP1* was associated to lactation persistency (Bissonnette, 2018).

Adaptation (20%) and functional traits (20% of the genes) might indicate the inherited resilience of the Modenese from the Podolian
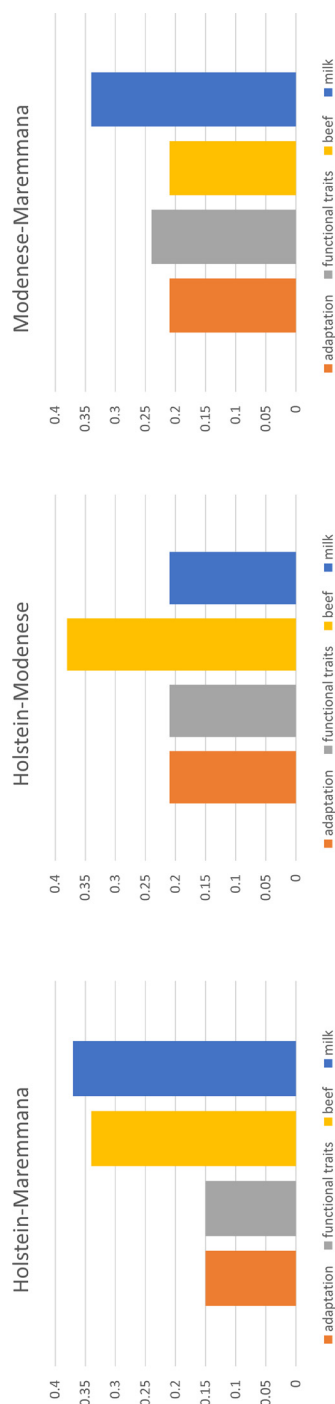
ancestors. Three genes - *TMED4, ERAP2* and *LNPEP* - encode the genomic divergence between zebu and taurine cattle (Huang et al., 2012; Porto-Neto et al., 2013).

Three genes are reported to be involved in disease resistance: *SLC4A4* and *STK38* to clinical mastitis (Sahana et al., 2014; Brand et al., 2011), *ERAP1* to bovine tuberculosis (Blanco et al., 2017). Among the genes influencing functional traits, *DDX46, ANKRD11, DDX42* and *SMARCD2* play a role in fertility (Hatzirodos et al., 2014; Moore et al., 2016; Killeen et al., 2014); *NRXN1* affects SCC (Raven et al., 2016) while *UST* peri-natal mortality (Purfield et al., 2015).

Two of the mentioned genes, *PDK2* and *SPP1*, are in proximity of two SNPs detected also in the comparison Modenese-Maremmana (Hapmap27072-BTC-033816 and Hapmap27503-BTC-033786). The influence on feed efficiency and on lactation persistency of the two genes respectively might be the results of the selection of the Modenese for the dual-purpose attitude.

4.3. Modenese-Maremmana

The 76 SNPs differentiating Maremmana from Modenese (Supplementary Table S3) are located in proximity of 122 potential candidate genes, of which 39 are known to play an important role in cattle (Table 3). The majority of them influence dairy traits (Fig. 2) as expected from comparing a suckler cow with a dairy one: *CSF2RB, NCF4, RGL1* and *VSTM1* were associated to milk yield (Raven et al., 2016; Yodklaew et al., 2017; Bentley et al., 2015), *EPN1, LSM2, SMAP1* and *NARS* to milk quality (Suchocki et al., 2016; Li et al., 2016), *CCND2, CRACR2A, FGF23, FGF6* and *SPP1* to lactation persistency (Do et al., 2017; Bissonnette, 2018). Almost all the genes with evident role in beef traits *ATP8A1, DCAF16, LCORL, FAM184B, HMCN1, PKD2* and *TMSB4X* were associated to daily gain and feed efficiency (Taye et al., 2017a; Zhang et al., 2016a; Abo-Ismail et al., 2014; Chen et al., 2011) confirming that breeding strategies for the Modenese aimed to produce a dual-purpose breed; only one gene, *MDH2* was associated to marbling (Shin and Chung, 2016).

The six genes here classified as adaptation genes have been all associated to disease resistance, suggesting that the Maremmana might have preserved alleles favorable to resilience more than the Modenese, which was selected for production improvement. More specifically, *EHMT2, TEAD1* and *TIGAR* were associated to trypanotolerance (Fisher et al., 2007; Kim et al., 2017); *IFNAR1* and *IFNGR2* to immune response after infections with Micobacterium bovis (Killick et al., 2011); *TLR7* and *TLR8* to Bovine herpesvirus (Rensetti et al., 2016) and *CCDC91* to paratuberculosis (Kupper, 2014). Similarly, it is likely that the Maremmana has preserved favorable alleles in genes involved in reproduction and mastitis resistance, as suggested by *FYN, GART, GSTA2, NLRP9, PARP11* and *SLC5A11*, which have been associated to fertility (Pinto de Melo et al., 2017; Fritz et al., 2013; Pinto de Melo et al., 2017; Cochran et al., 2013; Han and Penagaricano, Y. 2016; Hayashi et al., 2017), by *LGI2* associated to longevity (Zhang et al., 2016a) and by *NPFFR2, CDKN1B,* and *UBA6*, associated to mastitis susceptibility and SCC (Sahana et al., 2014; Abdel-Shafy et al., 2014).

4.4. GO analysis

The GO (Table 4) did not offer additional clues to elucidate the role of the putative candidate genes in shaping the breeds under investigation. The most represented categories were, in fact, the same for each of the three breed pairs: "cellular process" is the cell cycle expression; "metabolic process" includes both biosynthetic and catabolic processes; "binding (GO:0005488)" implements interactions between molecules at specific sites; "catalytic activity (GO:0003824)" provides catalysis of biochemical reactions at physiological temperatures; "cell part (GO:0044464)" represents the basic structural and functional unit of all organisms and "organelle (GO:0043226)" includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton.

Fig. 2. Frequency of the putative candidate genes influencing adaptation, functional traits, beef and milk traits, for each breed pair.

Table 4
Gene Ontology classification of genes in proximity of the SNPs significantly different in the breed comparisons.

	Holstein Maremmana		Holstein Modenese		Modenese Maremmana	
	n. genes	%	n. genes	%	n. genes	%
Biological process						
cellular process (GO:0009987)	53	32.10%	25	28.40%	51	29.70%
metabolic process (GO:0008152)	44	26.70%	20	22.70%	41	23.80%
biological regulation (GO:0065007)	17	10.30%	12	13.60%	18	10.50%
response to stimulus (GO:0050896)	12	7.30%	8	9.10%	18	10.50%
localization (GO:0051179)	11	6.70%	8	9.10%	13	7.60%
cellular component organization or biogenesis (GO:0071840)	9	5.50%	6	6.80%	8	4.70%
developmental process (GO:0032502)	8	4.80%	3	3.40%	9	5.20%
multicellular organismal process (GO:0032501)	5	3.00%	4	4.50%	7	4.10%
immune system process (GO:0002376)	2	1.20%	1	1.10%	3	1.70%
other	4	2.40%	1	1.10%	4	2.20%
Molecular function						
binding (GO:0005488)	37	46.80%	15	37.50%	38	38.40%
catalytic activity (GO:0003824)	29	36.70%	13	32.50%	35	35.40%
transporter activity (GO:0005215)	6	7.60%	7	17.50%	10	10.10%
signal transducer activity (GO:0004871)	3	3.80%	3	7.50%	6	6.10%
other	1	1.30%	1	2.50%	10	10.10%
Cellular components						
cell part (GO:0044464)	35	43.80%	16	39.00%	28	35.90%
organelle (GO:0043226)	23	28.80%	10	24.40%	19	24.40%
membrane (GO:0016020)	6	7.50%	7	17.10%	13	16.70%
macromolecular complex (GO:0032991)	13	16.30%	7	17.10%	13	16.70%
extracellular region (GO:0005576)	2	2.50%	1	2.40%	3	3.80%
other	1	1.30%	1	2.40%	4	5.10%

The evidence that these categories play a role in vary basic cell functions might contribute to further support conservation actions.

5. Conclusions

Through the present study, we described some genetic differences between Holstein and two local endangered breeds of different production purposes, and tried to provide new data supporting the sustainable use of animal genetic resources and the planning of conservation actions.

In general, comparison of Holstein with either of the two endangered breeds showed that most of the candidate genes differentiating the breeds have been previously associated with productive (dairy or meat) traits in cattle, in agreement with higher selection intensity for increased productivity applied to the Holstein breed. Differences between Holstein and Maremmana rely equally on dairy and beef traits. On the other hand, genetic differences between Holstein and Modenese could be largely attributed to genes with known associations to adaptation and functional traits, and in addition to genes associated with meat production, in accordance with the dual purpose of Modenese. However, when compared with Maremmana, the Modenese appear different not only in dairy traits, but also in adaptation and functional traits, suggesting that this breed might have lost some features of the Podolian ancestors as consequence of the selection practices.

Conflict of interest statement

There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2018.07.011.

References

Abdel-Shafy, H., Bortfeldt, R.H., Tetens, J., Brockmann, G.A., 2014. Single nucleotide polymorphism and haplotype effects associated with somatic cell score in German Holstein cattle. Gen. Sel. Evol. 46, 35. https://doi.org/10.1186/1297-9686-46-35.

Abo-Ismail, M.K., Van der Voort, G., Squires, J.J., Swanson, K.C., Mandell, I.B., Liao, X., Stothard, P., Moore, S., Plastow, G., Miller, S.P., 2014. Single nucleotide polymorphisms for feed efficiency and performance in crossbred beef cattle. BMC Genet. 15, 14.

AIA, 1981. Milk Recording Activity: Official Statistics Associazione Italiana Allevatori. Roma, Italy.

Aulchenko, Y.S., Ripke, S., Isaacs, A., van Duijn, C.M., 2007. GenABEL: an R package for genome-wide association analysis. Bioinformatics 23, 1294–1296.

Bentley, P.A., Wall, E.H., Dahl, G.E., McFadden, T.B., 2015. Responses of the mammary transcriptome of dairy cows to altered photoperiod during late gestation. Physiol. Genomics 47, 488–499.

Bertolini, F, Galimberti, G., Calo, D.G., Schiavo, G., Matassino, D., Fontanesi, L., 2015. Combined use of principal component analysis and random forests identify population-informative single nucleotide polymorphisms: application in cattle breeds. J. Anim. Breed. Genet. 132, 346–356.

Bissonnette, N., 2018. Genetic association of variations in the osteopontin gene (SPP1) with lactation persistency in dairy cattle. J. Dairy Sci. 101, 456–461.

Blanco, F.C, Soria, M.A., Klepp, L.I., Bigi, F., 2017. ERAP1 and PDE8A are downregulated in cattle protected against Bovine Tuberculosis. J. Mol. Microbiol. Biotechnol. 27, 237–245.

Boettcher, P.J., Hoffmann, I., Baumung, R., Drucker, A.G., McManus, C., Berg, P., Stella, A., Nilsen, L.B., Moran, D., Naves, M., Thompson, M.C., 2015. Genetic resources and genomics for adaptation of livestock to climate change. Front. Genet. 5, 461. https://doi.org/10.3389/fgene.2014.00461.

Bonin, A., Nicole, F., Pompanon, F., Miaud, C., Taberlet, P., 2007. Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. Conserv. Biol. 21, 697–708.

Brand, B., Hartmann, A., Repsilber, D., Griesbeck-Zilch, B., Wellnitz, O., Kühn, C., Ponsuksili, S., Meyer, H.D., Schwerin, M., 2011. Comparative expression profiling of E. coli and S. aureus inoculated primary mammary gland cells sampled from cows with different genetic predispositions for somatic cell score. Gen. Sel. Evol. 43, 24.

Cesar, A.S.M., Regitano, L.C.A., Mourão, G.B., Tullio, R.R., Lanna, D.P.D, Nassu, R.T., Mudado, M.A., Oliveira, P.S.N., do Nascimento, M.L., Chaves, A.S., Alencar, M.M., Sonstegard, T.S., Garrick, D, Reecy, J.M., Coutinho, L.L., 2014. Genome-wide association study for intramuscular fat deposition and composition in Nellore cattle. BMC Genet. 5, 39.

Chen, Y., Gondro, C., Quinn, K., Herd, R.M., Parnell, P.F., Vanselow, B., 2011. Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Anim. Genet. 42, 475–490.

CNR, 1983. Atlante Etnografico Delle Popolazioni Bovine Alleviate in Italia. Consiglio Nazionale delle Ricerche, Roma, Italy.

Cochran, S.D., Cole, G.B., Null, D.J., Hansen, P.J., 2013. Discovery of single nucleotide polymorphisms in candidate genes associated with fertility and production traits in Holstein cattle. BMC Genet. 14, 49.. http://www.biomedcentral.com/1471-2156/14/49.

Cohen-Zinder, M., Asher, A., Lipkin, E., Feingersch, R., Agmon, R., Karasik, D., Brosh, A.,

Shabtay, A., 2016. FABP4 is a leading candidate gene associated with residual feed intake in growing Holstein calves. Physiol. Genomics 48, 367–376.

Cole, J.B., Wiggans, G.R., Ma, L., Sonstegard, T.S., Lawlor Jr, T.J., Crooker, B.A., Van Tassell, C.P., Yang, J., Wang, S., Matukumalli, L.K., Da, Y., 2011. Genome-wide association analysis of thirty production, health, reproduction and body conformation traits in contemporary U.S. Holstein cows. BMC Genomics 12, 408.

Curi, R.A., Palmieri, D.A., Suguisawa, L., de Oliveira, H.N., Silveira, A.C., Lopes, C.R., 2006. Growth and carcass traits associated with GH1/Alu I and POU1F1/Hinf I gene polymorphisms in Zebu and crossbred beef cattle. Genet. Mol. Biol. 29, 56–61.

de Almeida Santana, M.H., Oliveira Junior, G.A., Cesar, A.S., Freua, M.C., da Costa Gomes, R., da Luz E Silva, S., Leme, P.R., Fukumasu, H., Carvalho, M.E., Ventura, R.V., Coutinho, L.L., Kadarmideen, H.N., Ferraz, J.B., 2016. Copy number variations and genome-wide associations reveal putative genes and metabolic pathways involved with the feed conversion ratio in beef cattle. J. Appl. Genet. 57, 495–504 Epub 2016 Mar 21.

de Almeida Santana, M.H., Ventura, R.V., Utsunomiya, Y.T., Neves, H.H.R., Alexandre, P.A., Oliveira Junior, G.A., Gomes, R.C., Bonin, M.C., Coutinho, L.L., Garcia, J.F., Silva, S.L., Fukumasu, H., Leme, P.R., Ferraz, J.B.S., 2015. A genome wide association mapping study using ultrasound scanned information identifies potential genomic regions and candidate genes affecting carcass traits in Nellore cattle. J. Anim. Breed. Genet. 132, 420–427.

Do, D.N., Bissonnette, N., Lacasse, P., Miglior, F., Sargolzaei, M., Zhao, X., Ibeagha-Awemu, E.M., 2017. Genome-wide association analysis and pathways enrichment for lactation persistency in Canadian Holstein cattle. J. Dairy Sci. 100, 1955–1970. https://doi.org/10.3168/jds.2016-11910.

FAO, 2002. Organic Agriculture and Genetic Resources for Food and Agriculture. Corporate Document Repository, http://www.fao.org/docrep/005/AC784E/ac784e-04.htm, retrieved on 1 dec 2017.

FAO, 2000. World Watch List for Domestic Animal Diversity, third ed. Roma, Italy.

Fisher, P., Hedeler, C., Wolstencroft1, K., Hulme, H., Noyes, H., Kemp, S., Stevens, R., Brass, A., 2007. A systematic strategy for large-scale analysis of genotype–phenotype correlations: identification of candidate genes involved in African trypanosomiasis. Nucleic Acids Res. 35, 5625–5633.

Fritz, S., Capitan, A., Djari, A., Rodriguez, S.C., Barbat, A., Baur, A., Grohs, C., Weiss, B., Boussaha, M., Esquerre, D., Klopp, C., Rocha, D., Boichard, D., 2013. Detection of haplotypes associated with prenatal death in dairy cattle and identification of deleterious mutations in GART, SHBG and SLC37A2. PLoS ONE 8, e65550. https://doi.org/10.1371/journal.pone.0065550.

Gautier, M., Flori, L., Riebler, A., Jaffrézic, F., Laloé, D., Gut, I., Moazami-Goudarzi, K., Foulley, J.L., 2009. A whole genome Bayesian scan for adaptive genetic divergence in West African cattle. BMC Genomics 10, 550.

Han, Y., Peñagaricano, F., 2016. Unravelling the genomic architecture of bull fertility in Holstein cattle. BMC Genet. 17, 143.

Hatzirodos, N., Hummitzsch, K., Irving-Rodgers, H.F., Harland, M.L., Morris, S.E., Rodgers, R.J., 2014. Transcriptome profiling of granulosa cells from bovine ovarian follicles during atresia. BMC Genomics 15, 40.

Höglund, J.K., Buitenhuis, B., Guldbrandtsen, B., Lund, M.S., Sahana, G., 2016. Genome-wide association study for female fertility in Nordic Red cattle. BMC Genet. 16, 110.

Hayashi, K-G., Hosoe, M., Kizaki, K., Fujii, S., Kanahara, H., Takahashi, T., Sakumoto, R., 2017. Differential gene expression profiling of endometrium during the mid-luteal phase of the estrous cycle between a repeat breeder (RB) and non-RB cows. Reprod. Biol. Endocrinol. 15, 20.

Hiemstra, S.J., de Haas, Y., Mäki-Tanila, M., Gandini, G., 2010. Development of policies and strategies for self-sustaining breeds. Local Cattle Breeds in Europe. https://doi.org/10.3920/978-90-8686-697-7. ISBN: 978-90-8686-.

Huang, W., Nadeem, A., Zhang, B., Babar, M., Soller, M., Khatib, H., 2012. Characterization and comparison of the leukocyte transcriptomes of three cattle breeds. PLoS ONE 7, e30244.

Jiang, L., Liu, X., Yang, J., Wang, H., Jiang, J., Liu, L., He, S., Ding, X., Liu, J., Zhang, Q., 2014. Targeted resequencing of GWAS loci reveals novel genetic variants for milk production traits. BMC Genomics 15, 1105.

Karisa, B.K., Thomson, J., Wang, Z., Bruce, H.L., Plastow, G.S., Moore, S.S., 2013. Candidate genes and biological pathways associated with carcass quality traits in beef cattle. Can. J. Anim. Sci. 93, 295–306.

Islam, K.K., 2009. Association Analyses of SNPs in Candidate Genes with Body Fat Deposition and Carcass Merit Traits in Beef Cattle. A thesis submitted for the degree of Master in Animal Science. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, USA.

Kijas, J.W., Lenstra, J.A., Hayes, B., Boitard, S., Porto Neto, L.R., San Cristobal, M., et al., 2012. Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. PLoS Biol. 10. https://doi.org/10.1371/journal.pbio.1001258.

Killeen, A.P., Morris, D.G., Kenny, D.A., Mullen, M.P., Diskin, M.G., Waters, S.M., 2014. Global gene expression in endometrium of high and low fertility heifers during the mid-luteal phase of the estrous cycle. BMC Genomics 15, 234.

Killick, K.E., Browne, J.A., Park, S.D., Magee, D.A., Martin, I., Meade, K.G., Gordon, S.V., Gormley, E., O'Farrelly, C., Hokamp, K., MacHugh, D.E., 2011. Genome-wide transcriptional profiling of peripheral blood leukocytes from cattle infected with Mycobacterium bovis reveals suppression of host immune genes. BMC Genomics 12, 611.

Kim, S.G., Ka, S., Ha, J.W., Kim, J., Yoo, D.A., Kim, K., Lee, H.K., Lim, D., Cho, S., Hanotte, O., Mwai, O.A., Dessie, T., Kemp, S., Oh, S.J., Kim, H., 2017. Cattle genome-wide analysis reveals genetic signatures in trypanotolerant N'Dama. BMC Genomics 18, 371.

Kim, E.S., Rothschild, M.F., 2014. Genomic adaptation of admixed dairy cattle in East Africa. Front. Genet. 5, 443.

Kupper, J.D., 2014. Genetic and Phenotypic Aspects of Infection with Mycobacterium Avium ssp. Paratuberculosis in German Holstein Cows. A thesis submitted for the degree of Doctor of Philosophy, to the Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management. Justus-Liebig-University Gießen, Germany.

Lee, S.H., Choi, B.H., Lim, D., Gondro, C., Cho, Y.M., Dang, C.G., Sharma, A., Jang, G.W., Lee, K.T., Yoon, D., Lee, H.K., Yeon, S.H., Yang, B.S., Kang, H.S., Hong, S.K., 2013. Genome-wide association study identifies major loci for carcass weight on BTA14 in Hanwoo (Korean Cattle). PLoS ONE 8, e74677. https://doi.org/10.1371/journal.pone.0074677.

Lemos, M.V., Chiaia, H.L., Berton, M.P., Feitosa, F.L., Aboujaoud, C., Camargo, G.M., Pereira, A.S., Albuquerque, L.G., Ferrinho, A.M., Mueller, L.F., Mazalli, M.R., Furlan, J.J., Carvalheiro, R., Gordo, D.M., Tonussi, R., Espigolan, R., Silva, R.M., de Oliveira, H.N., Duckett, S., Aguilar, I., Baldi, F., 2016. Genome-wide association between single nucleotide polymorphisms with beef fatty acid profile in Nellore cattle using the single step procedure. BMC Genomics 17, 213.

Li, C., Cai, W., Zhou, C., Yin, H., Zhang, Z., Loor, J.J., Sun, D., Zhang, Q., Liu, J., Zhang, S., 2016. RNA-Seq reveals 10 novel promising candidate genes affecting milk protein concentration in the Chinese Holstein population. Scientific Reports 6, 26813. https://doi.org/10.1038/srep26813.

Magalhães, A.F., de Camargo, G.M., Fernandes, G.A., Gordo, D.G., Tonussi, R.L., Costa, R.B., Espigolan, R., Silva, R.M., Bresolin, T., de Andrade, W.B., Takada, L., Feitosa, F.L., Baldi, F., Carvalheiro, R., Chardulo, L.A., de Albuquerque, L.G., 2016. Genome-wide association study of meat quality traits in Nellore cattle. PLoS One 11, e0157845.

Maretto, F., Ramljak, J., Sbarra, F., Penasa, M., Mantovani, R., Ivankovic, A., Bittante, G., 2012. Genetic relationships among Italian and Croatian Podolian cattle breeds assessed by microsatellite markers. Livest. Sci. 150, 256–264.

Mariasegaram, M., Chase Jr, C.C., Chaparro, J.X., Olson, T.A., Brenneman, R.A., Niedz, R.P., 2007. The slick hair coat locus maps to chromosome 20 in Senepol-derived Cattle. Animal Genet. 38, 54–59.

Mészáros, G., Eaglen, S., Waldmann, P., Sölkner, J., 2014. A genome wide association study for longevity in cattle. Open J. Genet. 4, 46–55.

Mi, H., Muruganujan, A., Thomas, P.D., 2013. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res. 41, D377–D386.

Moioli, B., Pilla, F., Ciani, E., 2015. Signatures of selection identify loci associated with fat tail in sheep. J. Anim. Sci. 93, 4660–4669.

Moioli, B., Steri, R., Marchitelli, C., Catillo, G., Buttazzoni, L., 2017. Genetic parameters and genome-wide associations of twinning rate in a local breed, the Maremmana cattle. Animal 11, 1660–1666.

Moore, S., 2015. Genetic control of dairy cow reproduction. A thesis submitted to the National University of Ireland for the degree of Doctor of Philosophy, Animal and Bioscience Research Department, Teagasc Moorepark. Fermoy, Co. Cork, Ireland.

Moore, S.G., Pryce, J.E., Hayes, B.J., Chamberlain, A.J., Kemper, K.E., Berry, D.P., McCabe, M., Cormican, P., Lonergan, P., Fair, T., Butler, S.T., 2016. Differentially expressed genes in endometrium and corpus luteum of Holstein cows selected for high and low fertility are enriched for sequence variants associated with fertility. Biol. Reprod. 94, 19.

Newman, J.H., Holt, T.N., Cogan, J.D., Womack, B., Phillips, J.A., Li, C., Kendall, Z., Stenmark, K.R., Thomas, M.J, Brown, R.D., Riddle, S.R., West, J.D., Hamid, R., 2015. Increased prevalence of EPAS1 variant in cattle with high-altitude pulmonary hypertension. Nat. Comm. 6, 6863. https://doi.org/10.1038/ncomms7863.

O'Gorman, G.M., Park, S.D.E., Hill, E.W., Meade, K.G., Coussens, P.M., Agaba, M., Naessens, J., Kemp, S.J., MacHugh, D.E., 2009. Transcriptional profiling of cattle infected with Trypanosoma congolense highlights gene expression signatures underlying trypanotolerance and trypano- susceptibility. BMC Genomics 10, 207.

Oldenbroek, K., 2007. Utilization and Conservation of Farm Animal Genetic Resources, first ed. Wageningen Academic Publishers, The Netherlands.

Pareek, C.S., Bøaszczyk, P., Dziuba, P., Czarnik, U., Fraser, L., Sobiech, P., Pierzchaøa, M., Feng, Y., Kadarmideen, H.N., Kuma, D., 2017. Single nucleotide polymorphism discovery in bovine liver using RNA-seq technology. PLoS ONE 12, e0172687. https://doi.org/10.1371/journal.pone.0172687.

Petrera, F., Catillo, G., Napolitano, F., Malacarne, M., Franceschi, P., Summer, A., Abeni, F., 2016. New insights into the quality characteristics of milk from Modenese breed compared with Italian Friesian. Ital. J. Anim. Sci. 15, 559–567. https://doi.org/10.1080/1828051X.2016.1222889.

Pinto de Melo, T., de Camargo, G.M.F., Galvão de Albuquerque, L., Carvalheiro, R., 2017. Genome-wide association study provides strong evidence of genes affecting the reproductive performance of Nellore beef cows. PLoS ONE 12, e0178551.

Pinto de Melo, T., 2015. Genome-Wide Association Study of Reproduction Trais in Nelore Cattle, Including Additional Phenotypic Information from Non-Genotyped Animals. A thesis submitted for the degree of Master in Animal Breeding and Genetics, to the Faculdade de Ciências Agrárias e Veterinárias Unesp. Câmpus, de Jaboticabal, Brazil.

Porto-Neto, L.R., Sonstegard, T.S., Liu, G.E., Bickhart, D.M., Da Silva, M.V.B., Machado, M.A., Utsunomiya, Y.T., Garcia, J.F., Gondro, C., Van Tassell, C.P., 2013. Genomic divergence of zebu and taurine cattle identified through high-density SNP genotyping. BMC Genomics 14, 876.

Purfield, D.C., Bradley, D.G., Evans, R.D., Kearney, F.J., Berry, D.P., 2015. Genome-wide association study for calving performance using high-density genotypes in dairy and beef cattle. Gen. Sel. Evol. 47, 47.

Randhawa, I.A.S., Khatkar, M.S., Thomson, P.C., Raadsma, H.W., 2015. Composite Selection Signals for Complex Traits Exemplified Through Bovine Stature Using Multibreed Cohorts of European and African Bos Taurus. G3 (Bethesda) 5, 1391–1401. https://doi.org/10.1534/g3.115.017772.

Raven, L-A, Cocks, B.G., Kemper, K.E., Chamberlain, A.J., Vander Jagt, C.J., Goddard,

M.E., Hayes, B.J., 2016. Targeted imputation of sequence variants and gene expression profiling identifies twelve candidate genes associated with lactation volume, composition and calving interval in dairy cattle. Mamm. Genome 27, 81–97.

Rensetti, D., Marin, M., Quintana, S., Morán, P., Verna, A., Odeón, A., Pérez, S., 2016. Involvement of toll-like receptors 3 and 7/8 in the neuropathogenesis of bovine herpesvirus types 1 and 5. Res. Vet. Sci. 107, 1–7.

Rincon, G., Islas-Trejo, A., Castillo, A.R., Bauman, D.E., German, B.J., Medrano, J.F., 2012. Polymorphisms in genes in the SREBP1 signalling pathway and SCD are associated with milk fatty acid composition in Holstein cattle. J. Dairy Res. 79, 66–75.

SAS, 2007. SAS/STAT User's Guide, Version 9.1. 2007. SAS Institute Inc., Cary, NC, USA.

Sahana, G., Guldbrandtsen, B., Thomsen, B., Holm, L-E., Panitz, F., Brøndum, R.F., Bendixen, R., Lund, M.S., 2014. Genome-wide association study using high-density single nucleotide polymorphism arrays and whole-genome sequences for clinical mastitis traits in dairy cattle. J. Dairy Sci. 97, 7258–7275.

Santos-Biase, W.K.F., Biase, F.H., Buratini Jr, J., Balieiro, J., Watanabec, Y.F., Accorsia, M.F., Ferreira, C.R., Stranieri, P., Caetano, A.R., Meirelles, F.V., 2012. Single nucleotide polymorphisms in the bovine genome are associated with the number of oocytes collected during ovum pick up. Anim. Reprod. Sci. 134, 141–149.

Serão, N.V.L, González-Peña, D., Beever, J.E., Faulkner, D.B., Southey, B.R., Rodriguez-Zas, S.L., 2013. Single nucleotide polymorphisms and haplotypes associated with feed efficiency in beef cattle. BMC Genet. 14, 94.

Silva-Vignato, B., Coutinho, L.L., Cesar, A.S.M., Poleti, M.D., Regitano, L.C.A., Balieiro, J.C.C., 2017. Comparative muscle transcriptome associated with carcass traits of Nellore cattle. BMC Genomics 18, 506.

Shin, S.C., Chung, E.R., 2016. Identification of differentially expressed genes between high and low marbling score grades of the longissimus lumborum muscle in Hanwoo (Korean cattle). Meat Sci 121, 114–118.

Suchocki, T., Wojdak-Maksymiec, K., Szyda, J., 2016. Using gene networks to identify genes and pathways involved in milk production traits in Polish Holstein dairy cattle. Czech J. Anim. Sci. 61, 526–538.

Taye, M., Lee, W., Jeon, S., Yoon, J., Dessie, T., Hanotte, O., Mwai, O.A., Kemp, S., Cho, S., Oh, S.J., Lee, H., Kim, H., 2017a. Exploring evidence of positive selection signatures in cattle breeds selected for different traits. Mamm. Genome 28, 528–541. https://doi.org/10.1007/s00335-017-9715-6.

Taye, M., Kim, J., Yoon, S.E., Lee, W., Hanotte, H., Dessie, T., Kemp, S., Mwai, O.A., Caetano-Anolles, K., Cho, S., Oh, S.J., Lee, H., Kim, H., 2017b. Whole genome scan reveals the genetic signature of African Ankole cattle breed and potential for higher quality beef. BMC Genet. 18, 11.

van den Berg, I., Fritz, S., Rodriguez, S., Rocha, D., Boussaha, M., Lund, M.S., Boichard, D., 2014. Concordance analysis for QTL detection in dairy cattle: a case study of leg morphology. Gen. Sel. Evol. 46, 31.

Wu, X., Fang, M., Liu, L., Wang, S., Liu, J., Ding, X., Zhang, S., Zhang, Q., Zhang, Y., Qiao, L., Lund, M.S., Su, G., Sun, D., 2013. Genome wide association studies for body conformation traits in the Chinese Holstein cattle population. BMC Genomics 14, 897.

Wu, Y., Fan, H., Wang, Y., Zhang, L., Gao, X., Chen, Y., Li, J., Ren, H.Y., Gao, H., 2014. Genome-Wide association studies using haplotypes and individual SNPs in Simmental cattle. PLoS ONE 9, e109330. https://doi.org/10.1371/journal.pone.0109330.

Xu, L., Bickhart, D.M., Cole, J.B., Schroeder, S.G., Song, J., Van Tassell, C.P., Sonstegard, T.S., Liu, G.E., 2014. Genomic signatures reveal new evidences for selection of important traits in domestic cattle. Mol. Biol. Evol. 32, 711–725.

Yodklaew, P., Koonawootrittriron, S., Elzo, M.A., Suwanasopee, T., Laodima, T., 2017. Genome-wide association study for lactation characteristics, milk yield and age at first calving in a Thai multibreed dairy cattle population. Agric. Natural Resour. 51, 223–230.

Zarek, C.M., Lindholm-Perry, A.K., Kuehn, L.A., Freetly, H.C., 2017. Differential expression of genes related to gain and intake in the liver of beef cattle. BMC Res. Notes 10, 1.

Zhang, Q., Guldbrandtsen, B., Thomasen, J.R., Lund, M.S., Sahana, G., 2016a. Genome-wide association study for longevity with whole-genome sequencing in 3 cattle breeds. J. Dairy Sci. 99, 7289–7298.

Zhang, W., Li, J., Guo, Y., Zhang, L., Xu, L., Gao, X., Zhu, B., Gao, H., Ni, H., Chen, Y., 2016b. Multi-strategy genome-wide association studies identify the DCAF16-NCAPG region as a susceptibility locus for average daily gain in cattle. Sci. Rep. 6, 3807. https://doi.org/10.1038/srep38073.

Zhao, F., McParland, S., Kearney, F., Du, L., Berry, D.P., 2015. Detection of selection signatures in dairy and beef cattle using high-density genomic information. Genet Sel Evol. 47, 49.